Full Paper

A Carbazole Derivative Protects Cells Against Endoplasmic Reticulum (ER) Stress and Glutathione Depletion

Hikari Miura^{1,2}, Katsura Takano³, Yasuko Kitao^{1,2}, Satoshi Hibino⁴, Tominari Choshi⁴, Rika Murakami⁵, Hiroto Suzuki⁵, Masashi Yamada⁶, Satoshi Ogawa^{1,2}, and Osamu Hori^{1,2,*}

¹Department of Neuroanatomy, Kanazawa University Graduate School of Medical Science, 13-1 Takara-Machi, Kanazawa, Ishikawa 920-8640, Japan

²CREST, JST (Japan Science and Technology), Tokyo, Japan

³Laboratory of Integrative Physiology in Veterinary Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Nakaku, Sakai, Osaka 599-8531, Japan

⁴Fukuyama University, Faculty of Pharmacy and Pharmaceutical Sciences, Sanzo 1, Gakuen-cho, Fukuyama, Hiroshima 729-0292, Japan

⁵Meiji Dairies Corporation, 2-10 Shinsuna 1-chome, Koto-ku, Tokyo 136-8908, Japan

⁶Taiho Pharmaceutical Co., Ltd., 1-27 Kandanishiki-cho, Chiyoda-ku, Tokyo 101-8444, Japan

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Abstract. Enhanced levels of intracellular stresses such as oxidative stress and endoplasmic reticulum (ER) stress are implicated in various neuropathological conditions including brain ischemia and neurodegeneration. During a search for compounds that regulate ER stress and ER stress-induced cell death, we identified a carbazole derivative 16-14 [9-(3-cyanobenzyl)-1,4-dimethylcarbazole] that protected against both ER stress and glutathione depletion. 16-14 suppressed tunicamycin (Tm)-induced cell death in both F9 Herp KO cells and PC12 cells, and its regulation of ER stress was associated with reduced levels of unfolded protein response (UPR) signaling. ER stress caused by overexpression of a fluorescent ER-resident protein, GFP-KDEL, was also attenuated by 16-14 without altering the expression levels of GFP-KDEL. 16-14 also prevented glutathione depletion-induced cell death caused by buthionine sulfoximine (BSO), but not likely via its anti-oxidative activity. Further analysis revealed that 16-14 suppressed increases in intracellular Ca^{2+} in response to thapsigargin (Tg). These results suggest that 16-14 may protect cells against different stresses via the maintenance of intracellular Ca^{2+} homeostasis. [Supplementary Fig. 1: available only at <http://dx.doi.org/10.1254/jphs.08136FP>]

Keywords: neuronal cell death, stress response, endoplasmic reticulum (ER), glutathione, neuroprotection

Introduction

Increased levels of intracellular stress contribute to various neuropathological conditions including brain ischemia and neurodegeneration (1). Administration of neurotoxins or overexpression of disease-related genes in neurons can cause mitochondrial damage and enhance oxidative stress. However, the endoplasmic reticulum (ER) may also play a role in these conditions. Secretory

proteins undergo post-translational modifications in the ER, which requires high levels of luminal Ca^{2+} and oxidative components. Exposure of cells to glucose starvation, inhibition of protein glycosylation, disturbance of Ca^{2+} homeostasis, or oxygen deprivation devastates ER environments and causes unfolded proteins to accumulate in the ER (ER stress). Eukaryotic cells, including neurons, respond to ER stress by activating a set of pathways known as the unfolded protein response (UPR) (2). The UPR is transmitted through the activation of ER-resident proteins, such as $\text{Ire1}\alpha/\beta$, PERK and ATF6, and the UPR-targeted genes include molecular chaperones, folding catalysts, ER-associated degradation

*Corresponding author. osamuh3@staff.kanazawa-u.ac.jp
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(ERAD) molecules, and antioxidants (2). However, if the protein load in the ER exceeds its folding capacity, cells tend to die, typically with apoptotic features (ER stress-induced cell death). Enhanced levels of ER stress and ER stress-induced cell death occur in brain ischemia, Alzheimer's disease, Parkinson's disease, and polyglutamine diseases (3, 4).

Herp is an UPR-dependent ubiquitin-like protein located in the ER of multiple cells, including neurons (5–7). Targeted disruption of the Herp gene renders F9 embryonic carcinoma cells vulnerable to ER stress (5), suggesting a protective role of Herp against ER stress. Using this and other cell lines, including rat pheochromocytoma PC12 cells, we evaluated the protective effect of 400 compounds against ER stress-induced cell death and found several ER stress-protective agents (8–10). We report here that a carbazole derivative, 16-14 (9-(3-cyanobenzyl)-1,4-dimethylcarbazole), protects PC12 cells against both ER stress and glutathione depletion. 16-14 suppressed ER stress caused by tunicamycin (Tm) or by overexpression of an ER-resident protein, GFP-KDEL. The regulation of ER stress by 16-14 was associated with reduced levels of UPR signaling. As 16-14 suppressed Ca^{2+} overload in response to thapsigargin (Tg), 16-14 may protect cells against different types of cellular stresses via mechanisms that include the maintenance of intracellular Ca^{2+} homeostasis.

Materials and Methods

Cell cultures and stress conditions

F9 Herp null cells were developed as previously described (5) and maintained in DMEM containing 20% FBS. PC12 cells and 293T cells were maintained in DMEM containing 5% FBS/5% horse serum and 10% FBS, respectively. ER stress was induced either by treating the cells with Tm (0.75–2 $\mu\text{g}/\text{ml}$; Sigma, St. Louis, MO, USA), an inhibitor of *N*-linked glycosylation, or brefeldin A (BFA) (1 $\mu\text{g}/\text{ml}$, Sigma), an inhibitor of protein transport from the ER to the Golgi apparatus, for 6–48 h or by transfecting GFP-KDEL cDNA, whose gene product accumulates in the ER (11). Oxidative stress was induced by exposing the cells to H_2O_2 (260 μM ; Nacalai Tesque, Kyoto) or to buthionine sulfoximine (BSO), a drug that causes depletion of intracellular glutathione (1 mM; Wako, Osaka). Compounds were added to the cells together with stressors or cells were also pre-incubated with compounds.

Compounds and cell viability assays

One hundred and three plant-derived compounds, including flavonoids and stilbenoids, and 300 synthetic

compounds, including dibenzoylmethane (DBM) derivatives, carbazole derivatives, and pyrimidine derivatives were analyzed for the protective effects against ER stress in F9 Herp null cells, as previously described (8). Carbazole derivatives: 16-1 [9-(3-carboxybenzyl)-1,4-dimethylcarbazole], 16-6 [9-(3-methylbenzyl)-1,4-dimethylcarbazole], 16-14 [9-(3-cyanobenzyl)-1,4-dimethylcarbazole], and 16-16 [9-(3-cyanobenzyl)-6-methoxy-1,4-dimethylcarbazole] were synthesized as previously reported (12). Dantrolene (Dan), carbazole, *N*-acetyl cysteine (NAC), Tm, Tg, bradykinin (BK), and nifedipine (Nif) were obtained from Sigma. Cell viability under conditions of ER stress or oxidative stress was measured using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay (Nacalai Tesque).

Northern blotting

PC12 cells were treated with each compound for 16 h, after which Tm (2 $\mu\text{g}/\text{ml}$) or BFA (2 $\mu\text{g}/\text{ml}$) was added to the cells for 6 h. Total RNA (10 $\mu\text{g}/\text{condition}$) isolated from the cells was subjected to Northern blotting using cDNA fragments specific for glucose-regulated protein 78 (GRP78), a molecular chaperone in the ER; C/EBP-homologous protein (CHOP), a mediator of ER stress-induced cell death (2); or β -actin as described previously (5).

Western blotting and immunostaining

293T cells were solubilized in lysis buffer containing 10 mM Tris, 1 mM EDTA, 150 mM NaCl, 1% SDS, 1 mM PMSF, 1 $\mu\text{g}/\text{ml}$ aprotinin, and 1 $\mu\text{g}/\text{ml}$ leupeptin and then subjected to Western blotting with an anti-KDEL antibody (Stressgen Bioreagents, Victoria, BC, Canada) that recognizes both GRP78 and GRP94, an anti- β -actin antibody (Sigma), and an anti-Herp antibody (5). Sites of primary antibody binding were visualized using alkaline phosphatase-conjugated secondary antibodies. Immunostaining of 293T cells was performed with an anti-GRP78 antibody (Stressgen Bioreagents) after fixing the cells. Cy3-labeled anti-rabbit IgG was used as secondary antibody.

Measurement of intracellular glutathione and detection of oxidative modifications of proteins

Total glutathione contents were measured using a glutathione assay kit (Cayman Chemical; Ann Arbor, MI, USA). To analyze the oxidative modification of proteins, PC12 cells were treated with BSO in the absence or presence of compounds for 16 h. Cells were harvested and whole cell lysates were obtained by incubating the cells in ice-cold Radio-Immunoprecipitation Assay (RIPA) buffer containing 10 mM Tris, 1 mM

EDTA, 150 mM NaCl, 1% NP40, 0.1% SDS, 0.2% deoxycholate, 1 mM PMSF, 1 μ g/ml aprotinin, and 1 μ g/ml leupeptin. Oxidative modification of proteins (protein carbonylation) was detected using the OxyBlot™ Protein Oxidation Detection Kit (Chemicon, Temecula, CA, USA), which is based on a two-step reaction, the derivatization of carbonylated proteins with dinitrophenylhydrazine (DNPH), and Western blotting with the anti-DNP antibody.

Measurement of intracellular Ca^{2+}

The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured by using the fluorescence indicator Fluo-3 AM in a 96 well scanning fluorometer, Fluoroskan Ascent (LabSystems, Helsinki, Finland), as previously described (13). In brief, PC12 cell suspensions (2×10^5 cells/well) were washed with Tyrode's solution (137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 0.2 mM NaH_2PO_4 , 12 mM $NaHCO_3$, 5.5 mM glucose) and then loaded with 5 μ M Fluo-3 AM for 30 min. After washing with Tyrode's buffer again, the cells were left for 30 min at RT. Compounds were added to the cells and baseline fluorescence of the well was measured in a volume of 50 μ l. Tg or BK was added to the cells and the signal was measured immediately. For calibration, the F_{max} signal was obtained by adding 20 μ l of 1% NP40. The F_{min} signal was obtained by adding 15 μ l of 0.1 M EGTA.

Statistical analysis

We used the Bonferroni/Dunn test following a one-way ANOVA.

Results

Prevention of Tm-induced cell death by carbazole derivatives in F9 Herp null cells

We previously reported that dantrolene, an antagonist of ryanodine receptors in the ER, significantly prevented Tm-induced cell death in F9 Herp null cells (8). Using this agent as a positive control, 103 plant-derived compounds and 300 synthetic compounds were screened in F9 Herp null cells treated with Tm. Treatment with Tm (0.8 μ g/ml) decreased viability to 40% (SD 5) of the control (non-stressed) cells at 48 h (Fig. 1AII). Addition of 9-benzyl-1,4-dimethylcarbazoles (16-1, 16-6, 16-14 or 16-16; Fig. 1AI) improved the viability of Tm-treated cells to levels similar to dantrolene (30 μ M) (Fig. 1AII). 16-14 and 16-16 had stronger protective effects than other 9-benzyl-1,4-dimethylcarbazoles (Fig. 1AII), suggesting that the existence of a 3-cyano group in the 9-benzyl group of the carbazole nucleus may enhance protective activity. To assess the role of the 9-benzyl

A

II

B

II

Fig. 1. The effect of carbazole derivatives on ER stress in F9 Herp null cells. AI and BI: structure of carbazole derivatives. AII and BII: protection of F9 Herp null cells against Tm-induced cell death. F9 Herp null cells (5×10^5 cells/condition) were treated with Tm (0.8 μ g/ml) in the absence or presence of compounds for 48 h. Cell viability was evaluated using the MTT assay. Values shown are means (S.D.) of 4 experiments. * $P < 0.01$, ** $P < 0.001$.

Fig. 2. The effect of 16-14 on the unfolded protein response (UPR). **A:** PC12 cells (5×10^5 cells/condition) were treated with Tm (0.75 $\mu\text{g/ml}$) in the presence or absence of compounds for 48 h. Cell viability was evaluated using the MTT assay. Values shown are means (S.D.) of 4 experiments. * $P < 0.001$. **B:** PC12 cells were treated with Tm (2 $\mu\text{g/ml}$) in the presence or absence of the indicated compounds for 6 h after pretreatment with each compound or vehicle (culture medium) for 16 h. Total RNA (10 μg /condition) was isolated and subjected to Northern blotting with specific probes against GRP78, CHOP, and β -actin. **C:** GFP-KDEL cDNA was transfected into 293T cells by lipofection and cultured for 48 h in the presence or absence of the indicated compounds. Western blotting (I) or immunostaining (II) was performed as described in the text. Arrows and arrowheads indicate the cells that do or do not express GFP-KDEL, respectively.

group of the carbazole nucleus, we analyzed the protective properties of 1,4-dimethylcarbazole (DMC) and carbazole in the same system. Both compounds were less effective at blocking Tm-induced cell death than 9-benzyl-1,4-dimethylcarbazoles (Fig. 1BI and II), suggesting that the 9-benzyl group of the carbazole nucleus may be important in this activity.

Regulation of ER stress by carbazole derivatives in PC12 cells

We also treated PC12 cells with Tm and measured cell viability as described above. Dantrolene, 16-14, and 16-16, but not carbazole, prevented Tm-induced cell death (Fig. 2A). To analyze the mechanism underlying the protective property of 16-14 against ER stress, PC12 cells were treated with Tm (2 $\mu\text{g}/\text{ml}$) in the presence or absence of dantrolene or 16-14 for 6 h, after pretreatment of the cells with each compound or vehicle (culture medium) for 16 h. The induction of GRP78, a target gene of both the ATF6 and Ire1-XBP1 pathways, and CHOP, a target gene of the PERK-eIF2 α pathway that responds to ER stress, were suppressed by dantrolene or 16-14 (Fig. 2BI) but not by carbazole (Fig. 2BII), suggesting that 16-14 improves an ER luminal environment that reduces the level of ER stress after treating the cells with ER stressors, as previously described (9). Cells showed similar responses when treated with BFA (data not shown).

The effect of 16-14 on ER stress caused by overexpression of GFP-KDEL

To further analyze the effect of 16-14 on different types of ER stress, GFP-KDEL cDNA (Fig. 2C), which encodes an ER-resident GFP, or vector alone [pcDNA3.1 (+); data not shown] was transiently transfected into 293T cells. More than 70% of the cells were transfected (data not shown); and transfection increased expression of GRP78, GRP94, and Herp proteins (the latter two molecules are targets of ATF6 and Ire1-XBP1 pathways) in Western blots (Fig. 2CI) or by immunostaining (Fig. 2CII). Treatment of the cells with dantrolene or 16-14 after transfection suppressed expression of both proteins without altering the expression levels of GFP-KDEL (Fig. 2CI and II).

The effect of 16-14 on oxidative stress

As carbazole is an anti-oxidant (14), we analyzed the protective capacity of 16-14 against oxidative stress using PC12 cells exposed to H₂O₂ [Supplementary Fig. 1 (Fig. S1): available at online version only] or BSO (Fig. 3A). Exposure to 260 μM H₂O₂ for 24 h decreased cell viability to 29% (S.D. 6) (Fig. S1). Although NAC, a precursor of glutathione, improved viability to 76%,

but dantrolene or 16-14 (Fig. S1) did not. Treatment with 1 mM BSO for 48 h decreased cell viability (61%, S.D. 7), and Dan and 16-14 improved cell viability to 81% (S.D. 6) and 83% (S.D. 7), respectively (Fig. 3A), without changing either total glutathione levels (Fig. 3C) or protein carbonyls (Fig. 3D). They also improved cell viability after treatment with both 1 mM BSO and 0.75 $\mu\text{g}/\text{ml}$ Tm for 24 h (Fig. 3B). These results suggest that the effect of 16-14 may be associated with the improved ER luminal environment, and not with the anti-oxidative property.

Suppression of Ca²⁺ overload by 16-14

As both ER stress and glutathione depletion can compromise Ca²⁺ homeostasis in PC12 cells (15, 16) and as dantrolene protected PC12 cells against both stresses (Fig. 1AII, Fig. 2A, and Fig. 3A), we hypothesized that 16-14 may contribute to the maintenance of Ca²⁺ homeostasis. The [Ca²⁺]_i was measured with the fluorescence indicator Fluo-3 AM. The basal [Ca²⁺]_i was not affected by 16-14, averaging 200–400 nM in Ca²⁺-containing medium (Fig. 4AI and BI) and 0–200 nM in Ca²⁺-free medium (data not shown). In contrast, the increase of [Ca²⁺]_i on addition of Tg, an inhibitor of the ER Ca²⁺-ATPase, but not BK, which stimulates Ca²⁺ release downstream of IP₃ production, was suppressed by both of 16-14 and Dan and, to a less extent, suppressed by Nif, a voltage-dependent L-type Ca²⁺-channel blocker (Fig. 4AI and II). Similar results were obtained in both Ca²⁺-containing-medium (Fig. 4AI and II) and Ca²⁺-free medium (data not shown).

Discussion

Carbazole and its derivatives are alkaloids produced in plants such as the *Murraya koengii* SPRENG (curry-leaf tree) and in other species of the genus *Murraya* or *Clausena* (17). Since the first reported isolation of murrayanine from *Murraya koengii* SPRENG (18), carbazole derivatives have continued to intrigue both chemists and biologists due to their structural features and bioactivities. They have anti-platelet/anti-inflammatory activity (19), anti-oxidative activity (20), anti-tumoral/anti-microbial activity (21), anti-mutagenic activity (22), and anti-fibrillar activity (23).

In this report, we demonstrated that a carbazole derivative, 16-14, protects cells against both ER stress and glutathione depletion. The presence of 9-benzyl groups, especially the 3-cyanobenzyl group, contributed to the protective properties of 16-14 against ER stress (Fig. 1A and B). Protection against ER stress/ER stress-induced cell death can be obtained in several ways: enhancement of the UPR activity or induction of the

Fig. 3. The effect of 16-14 on the glutathione depletion. A and B: PC12 cells (5×10^5 cells/condition) were treated with BSO (1 mM) alone for 48 h or with the combination of BSO (1 mM) and Tm (0.75 μg/ml) for 24 h in the presence or absence of compounds. Cell viability was evaluated using the MTT assay. Values shown are means (S.D.) of 4 experiments. * $P < 0.01$, ** $P < 0.001$. C: PC12 cells were treated or untreated with BSO in the absence or presence of the indicated compounds for 16 h, and total glutathione contents were measured. D: Detection of carbonylated proteins in whole cell lysates. PC12 cells (5×10^6 cells/condition) were pretreated with the 16-14 (40 μM) or with medium alone for 16 h, followed by a further incubation with BSO (1 mM) for 16 h in the presence or absence of compounds. Whole cell lysates were derivatized with DNPH and subjected to Western blotting with anti-DNP antibody as described in the text.

UPR-target genes (4); suppression of general protein synthesis (2); and improvement of the ER luminal environment, either through the maintenance of Ca^{2+} homeostasis or through the reduction of oxidative stress in the ER (9, 24). As the regulation of ER stress by 16-14 was associated with the reduced levels of expressions of the UPR-target genes in both Tm-treated cells and GFP-KDEL transfected cells, 16-14 may improve the ER luminal environment, which leads to reduced levels of ER stress. In fact, 16-14 suppressed increased $[\text{Ca}^{2+}]_i$ triggered by Tg to a similar level as dantrolene, suggest-

ing that 16-14 may protect cells by contributing to the maintenance of intracellular Ca^{2+} homeostasis. Although carbazole is an anti-oxidant (14), 16-14 failed to protect cells against H_2O_2 (Fig. S1). Similarly, our preliminary results indicated that 16-14 does not significantly affect general protein synthesis (data not shown). During analysis for the anti-oxidative property of 16-14, we found that both of dantrolene and 16-14 protected PC12 cells against glutathione depletion (Fig. 3A) without changing either total glutathione levels (Fig. 3C) or protein carbonyls (Fig. 3D). Furthermore, co-treatment

Fig. 4. The effect of 16-14 on the Ca²⁺ overload. PC12 cells (2×10^5 cells/well) were loaded with 5 μM Fluo-3 AM for 30 min, followed by incubation with the indicated compounds. After baseline fluorescence of the well was measured, thapsigargin (Tg) (A) or bradykinin (BK) (B) was added to the cells and the signal was measured immediately. Values shown are means (S.D.) of 4 experiments. * $P < 0.01$, ** $P < 0.001$. Nif: nifedipine, Dan: dantrolene.

of the cells with Tm lowered the viability in BSO-treated cells (Fig. 3B). These results are consistent with previous reports that depletion of GSH (reduced glutathione) increases $[Ca^{2+}]_i$ (25); and cycloheximide, a potent protein synthesis inhibitor, prevents BSO-induced cell death (26). It is, therefore, very likely that the ER plays an important role in the glutathione-depleted condition through its ability to maintain intracellular Ca²⁺ homeostasis.

It is currently unknown where 16-14 acts in the cells. The similar levels of the effects of 16-14 and dantrolene on the increase of $[Ca^{2+}]_i$ in response to Tg suggest that the function of 16-14 may be associated with the regulation of the expression and/or activity of ryanodine receptors in the ER. However, existence of the partial effect of Nif, although it is not significant, in the same conditions leads to another possibility that 16-14 may function near the voltage-dependent L- or N-type Ca²⁺ channels in the plasma membrane.

Perturbed intracellular Ca²⁺ homeostasis contributes to cell death directly by inducing or activating several cell death-related molecules, including Bax, P53, calpains, and caspases, or indirectly by enhancing intracellular stresses such as oxidative stress and ER stress (27). Enhanced levels of intracellular stresses in turn deteriorate intracellular Ca²⁺ homeostasis by increasing the influx of Ca²⁺ from the ER or plasma membrane or by inducing mitochondrial dysfunction. In this context,

it is important to find small, hydrophobic, and non-toxic compounds with Ca²⁺-regulating activity. Dan blocks neuronal death in models of both brain ischemia (24) and neurodegeneration (28, 29), but it has a severe hepatotoxicity (30). Our results suggest that 16-14 may be a candidate in the therapeutic treatment of neurological diseases. Our further studies are, therefore, aimed to identify the molecular target of 16-14 and to study the effect of 16-14 in vivo.

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References

- 1 Bossy-Wetzel E, Schwarzenbacher R, Lipton SA. Molecular pathways to neurodegeneration. *Nat Med.* 2004;10 Suppl:S2–S9.
- 2 Schröder M, Kaufman RJ. The Mammalian unfolded protein response. *Annu Rev Biochem.* 2005;74:739–789.

- 3 Lindholm D, Wootz H, Korhonen L. ER stress and neurodegenerative diseases. *Cell Death Differ.* 2006;13:385–392.
- 4 Tamatani M, Matsuyama T, Yamaguchi A, Mitsuda N, Tsukamoto Y, Taniguchi M, et al. ORP150 protects against hypoxia/ischemia-induced neuronal death. *Nature Med.* 2001;7:317–323.
- 5 Hori O, Ichinoda F, Yamaguchi A, Tamatani T, Taniguchi M, Koyama Y, et al. Role of Herp in the endoplasmic reticulum stress response. *Genes Cells.* 2004;9:457–469.
- 6 Kokame K, Agarwala KL, Kato H, Miyata T. Herp, a new ubiquitin-like membrane protein induced by endoplasmic reticulum stress. *J Biol Chem.* 2000;275:32846–32853.
- 7 Sai X, Kawamura Y, Kokame K, Yamaguchi H, Shiraishi H, Suzuki R, et al. Endoplasmic reticulum stress-inducible protein, Herp, enhances presenilin-mediated generation of amyloid beta-protein. *J Biol Chem.* 2002;277:12915–12920.
- 8 Takano K, Tabata Y, Kitao Y, Murakami R, Suzuki H, Yamada M, et al. Methoxyflavones protect cells against endoplasmic reticulum stress and neurotoxin. *Am J Physiol Cell Physiol.* 2007;292:C353–C361.
- 9 Takano K, Kitao Y, Tabata Y, Miura H, Sato K, Takuma K, et al. A dibenzoylmethane derivative protects dopaminergic neurons against both oxidative stress and endoplasmic reticulum stress. *Am J Physiol Cell Physiol.* 2007;293:C1884–C1894.
- 10 Tabata Y, Takano K, Ito T, Iinuma M, Yoshimoto T, Miura H, et al. Vaticanol B, a resveratrol tetramer, regulates endoplasmic reticulum stress and inflammation. *Am J Physiol Cell Physiol.* 2007;293:C411–C418.
- 11 Bannai H, Inoue T, Nakayama T, Hattori M, Mikoshiba K. Kinesin dependent, rapid, bi-directional transport of ER sub-compartment in dendrites of hippocampal neurons. *J Cell Sci.* 2004;117:163–175.
- 12 Cranwell PA, Saxton JE. A synthesis of ellipticine. *J Chem Soc.* 1962;3482–3487.
- 13 Toka FN, De Faundez IS, Gierynska M, Niemiałowski MG. The cytosolic free Ca^{2+} in ectromelia (mousepox) virus stimulated cytotoxic T lymphocytes. *Viral Immunol.* 1996;9:159–167.
- 14 Tang YZ, Liu ZQ. Free-radical-scavenging effect of carbazole derivatives on AAPH-induced hemolysis of human erythrocytes. *Bioorg Med Chem.* 2007;15:1903–1913.
- 15 Chan SL, Fu W, Zhang P, Cheng A, Lee J, Kokame K, et al. Herp stabilizes neuronal Ca^{2+} homeostasis and mitochondrial function during endoplasmic reticulum stress. *J Biol Chem.* 2004;279:28733–28743.
- 16 Doroshenko N, Doroshenko P. The glutathione reductase inhibitor carmustine induces an influx of Ca^{2+} in PC12 cells. *Eur J Pharmacol.* 2004;497:17–24.
- 17 Knölker HJ, Reddy KR. Isolation and synthesis of biologically active carbazole alkaloids. *Chem Rev.* 2002;102:4303–4427.
- 18 Chakraborty DP, Barman BK, Bose PK. On the constitution of murrayanine, a carbazole derivative isolated from *Murraya Koengii* Spreng. *Tetrahedron.* 1965;21:681.
- 19 Wu TS, Huang SC, Wu PL, Teng CM. Carbazole alkaloids from *Clausena excavata* and their biological activity. *Phytochemistry.* 1996;43:133–140.
- 20 Tachibana Y, Kikuzaki H, Lajis NH, Nakatani N. Comparison of antioxidative properties of carbazole alkaloids from *Murraya koenigii* leaves. *J Agric Food Chem.* 2003;51:6461–6467.
- 21 Pereira ER, Belin L, Sancelme M, Prudhomme M, Ollier M, Rapp M, et al. Structure-activity relationships in a series of substituted indolocarbazoles: topoisomerase I and protein kinase C inhibition and antitumoral and antimicrobial properties. *J Med Chem.* 1996;39:4471–4477.
- 22 Nakahara K, Trakoontivakorn G, Alzoreky NS, Ono H, Onishi-Kameyama M, Yoshida M. Antimutagenicity of some edible Thai plants, and a bioactive carbazole alkaloid, mahanine, isolated from *Micromelum minutum*. *J Agric Food Chem.* 2002;50:4796–4802.
- 23 Howlett DR, George AR, Owen DE, Ward RV, Markwell RE. Common structural features determine the effectiveness of carvedilol, daunomycin and rolitetracycline as inhibitors of Alzheimer beta-amyloid fibril formation. *Biochem J.* 1999;343:419–423.
- 24 Li F, Hayashi T, Jin G, Deguchi K, Nagotani S, Nagano I, et al. The protective effect of dantrolene on ischemic neuronal cell death is associated with reduced expression of endoplasmic reticulum stress markers. *Brain Res.* 2005;1048:59–68.
- 25 Doroshenko N, Doroshenko P. The glutathione reductase inhibitor carmustine induces an influx of Ca^{2+} in PC12 cells. *Eur J Pharmacol.* 2004;497:17–24.
- 26 Le Foll I, Duval DP. Programmed cell death induced by glutathione depletion in PC 12 cells is blocked by inhibitors of 12 lipoxygenase, but does not appear to be mediated through the formation of 12 HETE derivatives. *Free Radic Biol Med.* 2001;30:793–802.
- 27 Mattson MP. Calcium and neurodegeneration. *Aging Cell.* 2007;6:337–350.
- 28 Choi HJ, Kim SW, Lee SY, Moon YW, Hwang O. Involvement of apoptosis and calcium mobilization in tetrahydrobiopterin-induced dopaminergic cell death. *Exp Neurol.* 2003;181:281–290.
- 29 Ferreira E, Oliveira CR, Pereira C. Involvement of endoplasmic reticulum Ca^{2+} release through ryanodine and inositol 1,4,5-triphosphate receptors in the neurotoxic effects induced by the amyloid-beta peptide. *J Neurosci Res.* 2004;76:872–880.
- 30 Cornette M, Gillard C, Borlee-Hermans G. [Fatal toxic hepatitis associated with administration of dantrolene (author's transl).] *Acta Neurol Belg.* 1980;80:336–347. (text in French)